TREATMENT OF THIRD-DEGREE BURN WOUNDS IN ANIMAL SPECIMENS: ACELLULAR DERMIS OR PARTIAL-THICKNESS SKIN GRAFT

TRAITEMENT DES BRÛLURES DU 3ÈME DEGRÉ CHEZ L'ANIMAL: DERME ACELLULAIRE OU GREFFE SEMI-ÉPAISSE

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SUMMARY. Several dermal products have been introduced to substitute dermal tissues. In this study we review the effects of these products on repairing third-degree burn wounds and managing complications in animal specimens. Using an interventional approach, rats were randomly assigned to four groups (G1 to G4). Two wounds were created on the back of each rat. An open wound was left on the back of rats in G1; in G2, wounds were covered with a thick rat derived-ADM product and overlying thin skin graft; on G3 rats, similar third degree ulcers were made with one ulcer covered with harvested thin skin graft. In G4, ulcers were covered with a thin rat derived-ADM product and thin graft. Factors such as take rate, histopathological score, wound contracture and graft contracture were compared on the 7th, 15th, 21st and 30th day. Mean graft take rate on the 30th day in the thick ADM, thin ADM and graft group showed a significant difference (p=0.015). Histopathological score on the 30th day in the thin ADM, thick ADM and graft group showed no considerable difference. Mean graft take rate was significantly better in the thin ADM and graft group than in the thick ADM group. Wound contracture was significantly more severe in the thick ADM and control group than in the thin ADM and graft group.

Keywords: burns, acellular dermis, wound healing, skin substitute healing

RÉSUMÉ. Plusieurs produits ont été introduits dans le but de substituer le derme. Dans cette étude, nous avons étudié l'effet de ces produits sur la cicatrisation et la gestion des complications après brûlure expérimentale. Nous avons étudié 4 groupes (G1 à G4) de rats ayant subi deux brûlures du dos. Celles de G1 étaient laissées à l'air, G2 recevaient un Derme Artificiel Acellulaire (DAA) dérivé de rats épais recouvert d'une greffe fine, G3 recevaient une greffe conservée, G4 recevaient DAA fin et greffe fine. Le taux de prise de greffe, le score histologique, la rétraction de la brûlure et de la greffe ont été comparés à J7, J15, J21 et J30. A J30, les taux de prise de greffe étaient significativement différents entre G2, G3 et G4 (p=0,015), étant moins bons en cas d'utilisation de DAA en couche épaisse. De même, la rétraction était plus intense après utilisation de DAA en couche épaisse qu'en couche fine ou après greffe seule. Les résultats histologiques étaient comparables.

Mots-clés: brûlure, derme acellulaire, cicatrisation, substituts cutanés

Introduction

Plastic and burn surgeons have always found it challenging to achieve desirable cosmetic results when treating burns and traumas with full thickness skin damage. Tissue contracture, hypertrophic scarring and contour deformity are the most common complications that need to be addressed.[1-3]

These complications are often managed with dermal substitutes, particularly acellular dermal matrix (ADM) grafts which result in improved scar parameters.[4,5,6] These products provide a 3-dimensional matrix at the ulcer bed and help with focal vascularisation and fibroblast activity, which in turn leads to optimal and natural development of skin tissue.[7]

Several products have been introduced in different countries with varying clinical and experimental results. These include Alloderm, FlexHD, Neoform, Dermamatrix and Surgimend (human derived ADM), Strattice and Permacol (porcine-derived ADM).
In this study, we used an ADM product on rat specimens. This product was prepared by the "Iranian Tissue Product Company" at Tehran University of Medical Sciences.

Our study aimed to evaluate the effectiveness of our product, and its efficacy in supporting early skin grafts. We also compared thin and thick ADMs in terms of graft take and contracture rate.

Methods

The study was approved by the Ethics Committee of Iran University of Medical Sciences.

For ADM preparation, we used laboratory rat skin. Throughout

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the whole project, we followed national and international principles regarding caring for and using animals in experimental studies.

We chose male specimens weighing over 350 grams, and took the following steps to prepare the grafts:

1. Death was induced by CO2 inhalation;
2. The rat's back skin was shaved;
3. The skin was excised with power dermatome;
4. The skin was kept in a hypertonic saline incubator for 24 hours in order to facilitate separation of dermis and epidermis;
5. The epidermis and underlying adipose tissue were separated in a Class-100 Cleanroom under laminar hood;
6. The epidermis was treated with dispose, Triton X-100 and Pen Strep for a limited period of time;
7. The epidermis was rinsed multiple times;
8. It was then treated with cellular enzymatic lysis (Trypsin, Pen Strep and EDTA);
9. Final rinsing with PBS;
10. Lyophilisation;
11. Packaging and labelling for gamma irradiation;
12. Final sterilisation with gamma radiation;
13. Two types of ADM were prepared - thick ADM (0.6mm thickness) and thin ADM (0.4mm thickness).

At the end of preparation, the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was used to assess the immunogenicity of the grafts.

This is a colorimetric assay for assessing cell metabolic activity of tissues.

In the second stage of the study, twenty rats of similar race, age, gender and weight were anaesthetized by intramuscular injection of Xylazine 2% (15mg/kg) (Alfasan Inc., Woerden, the Netherlands) and Ketamine 10% (60mg/kg) (Alfasan Inc., Woerden, the Netherlands). Once anaesthetized, two full thickness wounds were created on the back of each rat using dermatome, Aesculap. Each area measured 2×2 cm.

The rats were randomly assigned to 4 groups (G1 to G4), each group including 5 rats.

In G1, the wounds were left open. In G2, the wounds were covered with thick ADM and overlaying thin skin autograft. In G3, harvested thin skin graft was used to cover the wounds. In G4, thin ADM and overlaying thin split-thickness skin graft (STSG) was used to cover them. The areas were then covered with vaseline and wet gauze.

Two rats from G1 and G2 died, which resulted in four wounds being excluded from our study. Hence, the number of open wounds (G1), thick ADM (G2), thin ADM (G3), skin graft (G4) and samples were 8, 8, 10 and 10 respectively.

The wounds were photographed on the 7th, 15th and 30th day with a digital camera (Canon, PowerShot SX200 IS, Tokyo, Japan) and several factors, such as graft take rate (GTR), residual wound area (RWA) and healed wound area (HWA) were analyzed using the ImageJ software programme (ver. 1.45, NIH, Maryland, USA). Two burn surgeons, blinded to the type of graft, clinically assessed the wounds in terms of graft colour and adherence.

The following formulas were used to measure wound contraction rate (WCR) and graft contraction (GCR):

• GTR = mean area of the graft take / mean area of wound X 100
• WCR = (wound area on 7th day – wound area on 30th day)/ wound area on 7th day X 100
• GCR = (graft area on 7th day – graft area on 30th day) / graft area on 7th day X 100

Wound biopsy was performed on the 15th and 30th day, and it was stained with haematoxylin and eosin stain (H&E stain or HE stain) (*Fig. 1*).

These were assessed with a histopathological scoring (HPS) system, reported in *Table I*.[8,9] *Table II* gives an example of a graft taken totally. The total score for signs of "healing" and "rejection" would be 0-6 and 0-10 respectively.

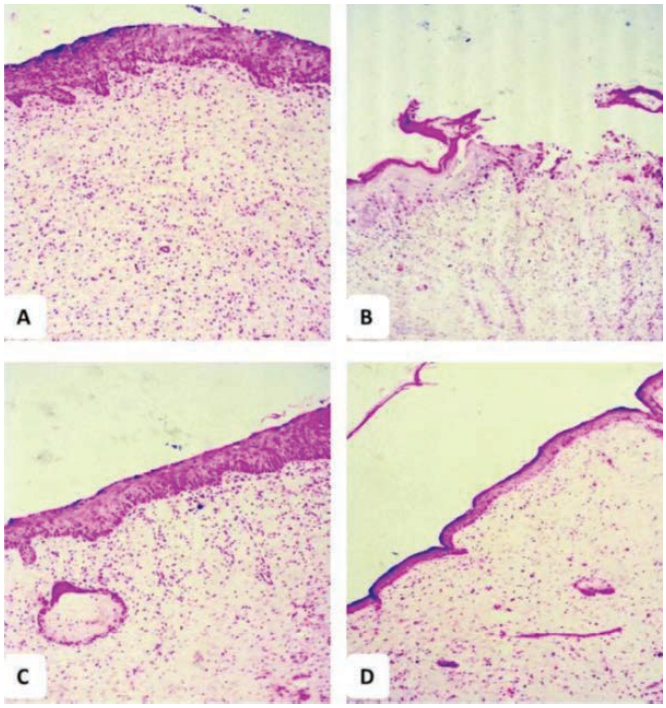

Fig. 1 - Histopathological results image. A: image on day 30 from skin in the graft group; B: image on day 30 from skin in the open wound group; C: image on day 30 from skin in the thick ADM group; D: image on day 30 from skin in the thin ADM group.

Table I - Criteria for the histopathological scoring (HPS) system

Signs		Scores
Healing and graft take	Epithelialization	0 = none 1 = complete
	Adherence	0 = <25% 1 = 25-75% 2 = 76-99% 3 = 100%
	Collagen pattern	0 = abnormal 1 = intermediate 2 = normal
		Total: 0-6
Graft rejection (Immunologic rejection)	Dermis and epidermis separation Fibroplasia Inflammatory cells Epidermal hyperplasia Eosinophilia	0 = presence of findings 1 = intermediate 2 = absence of findings
		Total: 0-10

Table II - Histopathological scoring: example of graft taken totally

Factor	Value	Score
Epithelialization	Complete	1
Adherence	Complete	3
Collagen pattern	Normal	2
Dermis and epidermis separation	None	2
Fibroplasia	None	2
Epidermal hyperplasia	None	2
Eosinophilia	None	2
Inflammatory cells	None	2
Overall score		16

A total score of 16 represents the best graft response, and a score of 0 indicates the worst graft response. Graft take also was clinically determined by an experienced burn surgeon.

These results were documented for all 4 groups on the 7th, 15th, 21st and 30th day.

ANOVA and T-testing were used to ensure that the results were normalised.

Results

Ten wounds were inflicted in each group. The Kolmogorov-Smirnov test was used to assess the variability of the data and ensure the normalised variation of the quantitative indices.

The difference in mean GTR among the groups is significant on the 7th, 21st and 30th day, as seen in *Table III*.

Table III - Binary comparison of mean graft take rate (GTR)

Group		7th day (%)	15th day (%)	21st day (%)	30th day (%)
Thick ADM	Mean±SD	29.18±3.57	52.88±4.4	69.9±3.74	62.4±4.24
	N	8	8	8	8
Thin ADM	Mean±SD	88.16±8.1	83.9±1.24	96.02±6.48	99.74±2.54
	N	10	10	10	10
Graft	Mean±SD	89.9±8.4	77.4±2.6	95.9±7.5	100
	N	10	10	10	10
P value between the three groups*		0.001>	0.102	0.021	0.015
P value between thick ADM and thin ADM **		0.001>	0.001>	0.003	0.002
P value between thick ADM and graft**		0.001>	0.21	0.008	0.001
P value between thin ADM and graft**		0.84	0.62	0.89	.098

*ANOVA **T test

HPS is significantly different on the 7th and 15th day in all 4 groups. However, there is no significant difference in the results of the binary comparison of G2, G3 and G4. There is a remarkable difference in the binary comparison of the results of G1 with each of the other three groups (*Table IV*).

GCR and GWR are somewhat similar in the binary comparison between the groups. However, a significant difference is seen for these two factors in the binary comparison of G1 and each of the other groups.

Reviewing WCR in G1 on the 21st and 30th day shows a significant difference ($p<0.001$). However, the other groups did not follow the same trend ($p>0.05$) (*Table V*).

Discussion

The optimal surgical management for burn wounds has been a subject of constant debate. The most crucial factor to consider is the depth of the wound. The standard treatments are early excision and grafting with a partial thickness skin graft, which has a thin dermis layer. This often leads to a slightly dipped and sunken appearance of the grafted area, which may eventually require cosmetic surgery. The thicker the dermal layer of the skin graft, the lower the rate of wound dip and contracture. Contracture could also be avoided by using another layer between the ulcer bed and the graft; hence, the introduction of acellular dermal matrices.

Table IV - Comparison of histopathological scores (HPS)

Group		15th day	30th day
Thick ADM	Mean±SD	9.2±3.96	9±2.4
	N	8	8
Thin ADM	Mean±SD	10.7±4.3	9.1±1.4
	N	10	10
Graft	Mean±SD	11.2±3.2	10.6±1.6
	N	10	10
Open wound	Mean±SD	3.5±1.2	5.7±3.9
	N	10	10
P value between the four groups*		0.002	0.003
P value between thick ADM and thin ADM**		0.74	0.88
P value between thick ADM and graft**		0.53	0.76
P value between thin ADM and graft**		0.80	0.69
P value between open wound and thick ADM **		0.001>	0.008
P value between open wound and graft**		0.001>	0.007
P value between open wound and thin ADM **		0.001>	0.001>

*ANOVA **T test

Table V - Comparison of WCR and GCR

Group		Wound contraction (21st day)	Wound contraction (30th day)	Graft contraction (21st day)	Graft contraction (30th day)
Thick ADM	Mean±SD	61.2±14.7	67.3±25.2	56.4±23.1	27.6±19.4
	N	8	8	8	8
Thin ADM	Mean±SD	46.7±19.3	45.6±9.8	41.3±17.9	38.1±15.4
	N	8	10	10	10
Graft	Mean±SD	29.6±12.8	37.8±17.9	22.6±13.5	30.6±14.9
	N	8	10	10	10
Open wound	Mean±SD	87.2±7.7	5.8±6.99	-	-
	N	8	10	-	-
P value between groups*		0.001>	0.001>	0.174	0.482
P value between thick ADM and thin ADM**		0.169	0.044	0.289	0.301
P value between thick ADM and graft**		0.039	0.207	0.108	0.696
P value between thin ADM and graft**		0.204	0.031	0.155	0.402
P value between open wound and thick ADM**		0.004	0.001>	-	-
P value between open wound and graft**		0.001>	0.001>	-	-
P value between open wound and thin ADM **		0.001>	0.001>	-	-

*ANOVA **T test

Dermis consists of a matrix composed of elastin, collagen and extrafibrillar matrix. This matrix is filled with different types of proteins which also support the fibroblasts, macrophages and adipocytes within the dermis.[1] Using a thin layer of dermis in the graft results in marked contracture and hypertrophic scarring. This occurs due to the conversion of fibroblasts into myofibroblasts fairly soon after grafting.[2,3] Acellular allograft dermal substitutes provide a 3-dimensional matrix which acts as scaffolding for the fibroblasts.[4,10,11,12,13] This improves graft adherence and reduces scar tissue, making them the ideal choice for treating deep and full skin thickness wounds.[14] The lesser risk of infection associated with acellular grafts makes them the graft of choice in stem-cell derived products which are expensive and time consuming to produce.

These grafts also require extensive disinfection processes.[4,15,16,17] Being expensive, the use of acellular grafts has remained limited.[7] In dermal wounds, using acellular dermal matrix under the graft leads to very good results, including better colour and skin elasticity, as well as limited scarring. Another advantage of ADM is that contour deformity is prevented.[14] Acellular dermal matrices are also reported to provide better results in repairing the cartilage matrix in joints[18] as well as in wound treatment in children.[19]

In our study, on day 7, GTR was lower in G2 (thick ADM) than in G3 (thin ADM) and G4 (graft). There was no significant difference between G3 and G4 in our study, but Pirayesh et al. indicate that the take rate of split thickness skin grafts was reduced with a one-stage procedure on top of Glyderm and Alloderm in porcine full thickness wounds.[20] We used one-stage procedures and sandwiched graft on thin and thick ADM.

On day 15, there was no noticeable difference in GTR among the three groups. However, binary comparison revealed that graft take was lower in G2 than in G3. On days 21 and 30, G2 showed the lowest rate of GTR, and no significant difference was seen between the other two groups.

Shrivastava et al. reported an 82% graft take rate on day 15 in rats with allogeneic alloderm used under the graft.[21] This is comparable to our study results for G3 (thin ADM). There was no meaningful statistical difference between G3 and G4. This confirms that ADM integrates with tissue and forms a permanent configuration, which is not triggering rejection as a foreign body.

We used a histopathological scoring system to determine the extent of integration of acellular dermis in the permanent structure of the wound bed, similar to other animal studies.[21,22,23] This proved that our product is similar, histologically. In many studies, collagen pattern was considered to be a positive score, and in others, the presence of collagen-elastin matrices was recognized as a sign of neodermis production.[24,25] In our study, HPS showed no considerable difference among the grafted groups (G2, G3 and G4). However, these groups had a strikingly higher score than the open wound group (G1). This again supports the fact that acellular dermis is not recognised as a foreign body. Cellular inflammation is much lower in the allogenic ADM products, particularly on day 15.

Wound contraction rate was significantly higher in the thick ADM group than in the other two groups. This usually leads to a second grafting attempt. No significant difference was seen between the thin ADM group and the graft group. The average size of the wound in G1 on the 21st day is smaller than in G4 and G3, revealing a smaller contracture rate in the thin ADM and the graft group. This is most likely due to the higher rate of graft take in both groups. A similar difference is seen among G1, G3 and G4 on days 21 and 30. Whilst WCR was not very different among the grafted groups at any stage, a significant difference was noted between the graft group and the open wound group.

Our findings are essentially similar to those of Van Zuijlen et al., where allogenic ADM showed a 49% lower rate of wound contracture.[26]

One of the limitations of our study is that the follow up was not long enough to assess long-term contracture rate. Using thick ADM in a two-stage procedure may be a viable graft option, which we would like to try in another study.

Overall, our study shows that using allogenic ADM products leads to better clinical outcome. Another advantage of this new acellular dermis is that it is cheaper and more readily available.[27] Hence, human use should be considered, following the necessary studies in the human phase.

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